Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Tupichinins B-D, three new spirostanol saponins from Tupistra chinensis rhizomes

Li-Mei Wei[a], Yang-Chang Wu[bc], Chin-Chau Chen[d], Pei-Wen Hsieh[e] & Wen-Bin Pan[a]

[a] Department of Applied Chemistry and Materials Science, Fooyin University, Kaohsiung 831, Taiwan, Republic of China

[b] Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 40402, Taiwan, Republic of China

[c] Natural Medicinal Products Research Center, China Medical University Hospital, Taichung 40402, Taiwan, Republic of China

[d] Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[e] Graduate Institute of Natural Products, Chang Gung University, Tao-Yuan333, Taiwan, Republic of China

Published online: 26 Nov 2013.

To cite this article: Li-Mei Wei, Yang-Chang Wu, Chin-Chau Chen, Pei-Wen Hsieh & Wen-Bin Pan (2014) Tupichinins B-D, three new spirostanol saponins from Tupistra chinensis rhizomes, Natural Product Research: Formerly Natural Product Letters, 28:2, 74-80, DOI: 10.1080/14786419.2013.838240

To link to this article: http://dx.doi.org/10.1080/14786419.2013.838240

Natural Product Research, 2014
Vol. 28, No. 2, 74–80, http://dx.doi.org/10.1080/14786419.2013.838240

Tupichinins B–D, three new spirostanol saponins from *Tupistra chinensis* rhizomes

Li-Mei Wei[a], Yang-Chang Wu[bc], Chin-Chau Chen[d], Pei-Wen Hsieh[e] and Wen-Bin Pan[a]*

[a]*Department of Applied Chemistry and Materials Science, Fooyin University, Kaohsiung 831, Taiwan, Republic of China;* [b]*Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 40402, Taiwan, Republic of China;* [c]*Natural Medicinal Products Research Center, China Medical University Hospital, Taichung 40402, Taiwan, Republic of China;* [d]*Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China;* [e]*Graduate Institute of Natural Products, Chang Gung University, Tao-Yuan 333, Taiwan, Republic of China*

(*Received 1 May 2013; final version received 19 July 2013*)

Three new spirostanol saponins were isolated from the EtOAc fraction of methanol extract from *Tupistra chinensis* rhizomes. Based on the detailed analysis of their 1D and 2D NMR spectra and chemical evidence, their structures were determined as 1β-*O*-acetyl-spirost-5,25(27)-dien-3α-yl-*O*-β-D-glucopyranoside (**1**), (25*S*)-1β,2β,5β-trihydroxy-spirostane-3β-yl-*O*-β-D-glucopyranoside (**2**) and (25*S*)-1β,2β-dihydroxy-5β-spirostane-3β-yl-*O*-β-D-xylopyranoside (**3**), respectively.

Keywords: *Tupistra chinensis*; Liliaceae; spirostanol saponins; tupichinins B, C and D

1. Introduction

The genus *Tupistra* (Liliaceae) comprising about 26 species is mainly distributed in Asia, of which about 17 species are growing in the People's Republic of China, particularly in the south-western region (Hang & Li 1990; Yang et al. 2005). Kai-Kou-Jian, the Chinese name of rhizomes of *Tupistra chinensis* BAK., is used for the treatment of rheumatic diseases and snakebite in Chinese folk medicine (Jiangsu New Medical College 1985). Kai-Kou-Jian is a reputed folk medicine because of its power to markedly reduce carbuncles and to ameliorate pharyngitis (Zhan 1994). Some furostanol saponins isolated from *T. chinensis* rhizomes showed inhibition action against NO production (Xu et al. 2007) and against COX-2 production (Zou, Wang et al. 2007; Zou, Wu et al. 2007).

Phytochemical investigation on *T. chinensis* in the past 15 years has resulted in the isolation of some steroidal sapogenins and steroidal saponins (Cai et al. 2007; Guo et al. 2009; Liu, Guo, Xue, Cheng et al. 2012; Liu, Guo, Xue, Zhang et al. 2012; Pan et al. 2000a, 2000b, 2003, 2006, 2012; Wu et al. 2005; Xu et al. 2007; Zou et al. 2005, 2006, 2007, 2009). Previously, we reported the isolation and structural elucidation of several spirostanol sapogenins, flavonoids, lignans, a pregnane glycoside, a pregnane genin and other chemical constituents from the $CHCl_3$ fraction of methanol extract from *T. chinensis* rhizomes (Pan et al. 2000a, 2000b, 2003, 2006). On continuing the study of this plant, we have now isolated three new spirostanol saponins, namely tupichinins B, C and D (Figure 1), from the EtOAc fraction of methanol extract from *T. chinensis* rhizomes. This study reported the isolation and structural elucidation of three new spirostanol saponins by detailed analysis of their 1D and 2D NMR spectra and acid hydrolysis.

*Corresponding author. Email: sc008@fy.edu.tw

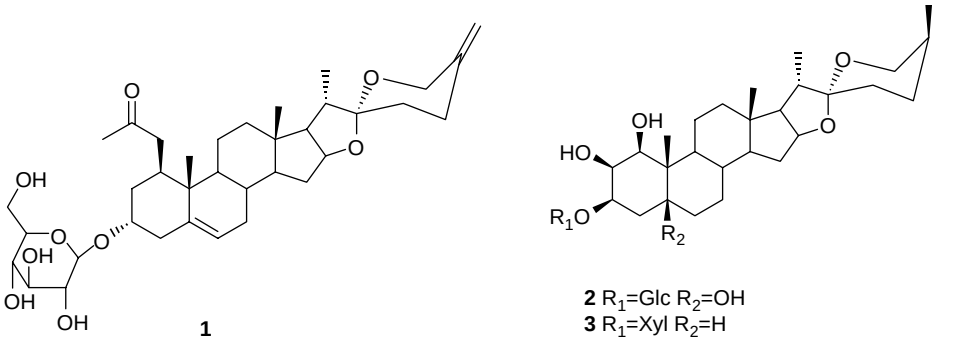

Figure 1. The chemical structures of compounds **1**–**3**.

2. Results and discussion

The EtOAc fraction of the methanol extract from the rhizomes of *T. chinensis* was chromatographed successively on silica gel to afford **1**, **2** and **3**.

Tupichinin B (**1**), an amorphous white solid, $[\alpha]_D^{24}$ −18° (*c* 0.004, MeOH), showed in the HRFABMS (positive mode) a pseudo-molecular $[M + Na]^+$ peak at *m/z* 655.3563 (calcd 655.3560), consistent with the molecular formula $C_{35}H_{52}O_{10}$, suggesting a spirostanol saponin skeleton with 10 degrees of unsaturation.

Unambiguous assignments for the ^1H and ^{13}C NMR signals were based on an analysis of the combination of distortionless enhancement by polarization transfer (DEPT), ^1H–^1H COSY, nuclear overhauser effect spectroscopy (NOESY), heteronuclear multiple quantum coherence and heteronuclear multiple bond correlation (HMBC) spectral data.

In the ^1H NMR spectrum in pyridine-d_5 of **1**, signals characteristic of the spirostanol saponin skeleton were observed. The ^1H NMR spectrum showed signals for two tertiary methyl groups at δ 0.88 (3H, s, Me-18) and 1.22 (3H, s, Me-19), a secondary methyl group at δ 1.14 (3H, d, $J = 7.0$ Hz, Me-21) and an anomeric proton at δ 4.98 (1H, d, $J = 7.5$ Hz). Two oxymethine proton resonances at δ 5.53 (1H, d, $J = 4.8$ Hz) and 4.42 (1H, br s) were assigned to H-1α and H-3β, respectively. Evidence for the presence of two double bonds at C-5 and C-25(27) came from an olefinic proton signal at δ 5.67 (1H, d, $J = 4.8$ Hz, H-6) and an exomethylene group at δ 4.86 and 4.89 (each 1H, s, H-27), respectively. Furthermore, an oxymethine proton resonance at δ 4.55 (1H, q-like, $J = 7.0$ Hz) was assigned to H-16. Two oxymethine proton resonances at δ 4.11 (1H, d, $J = 12.0$ Hz) and 4.50 (1H, d, $J = 12.0$ Hz) were assigned to H-26β and H-26α, respectively. The presence of an acetyl group in **1** was confirmed by ^1H NMR [δ 2.08 (3H, s)] and ^{13}C NMR [δ 171.5 (C═O) and 22.6 (Me)] spectra. The fully decoupled ^{13}C and DEPT nuclear magnetic resonance (NMR) spectra of **1** exhibited 35 carbon signals, which consisted of 4 methyls, 11 methylenes, 14 methines and 6 quaternary carbons. One carbonyl carbon at δ 171.5 (1-*O*-Ac); two vinylic carbons at δ 138.3 (C-5) and 126.7 (C-6); an anomeric carbon at δ 104.2 (C-1′ of glucose) and three methyl groups at δ 17.2 (C-18), 14.9 (C-19) and 15.8 (C-21) were confirmed in ^{13}C NMR spectra. Moreover, two oxygen-bearing carbon signals at δ 82.2 (CH) and 65.8 (CH_2) were assigned to the C-16 and C-26, respectively. Exomethylene carbon signals at δ 145.0 (C) and 109.9 (CH_2) were assigned to the C-25 and C-27, respectively.

In the HMBC spectrum (Figure S1), there were correlations between H-1α and C-2, C-9, C-10, C-19 and C═O (1-*O*-Ac). These findings supported the placement of an acetyl group on C-1 position. In the NOESY spectrum, the anomeric proton signal at δ 4.98 (H-1′ of glucose) showed correlations with the proton signals at 4.11 (1H, d, $J = 9.6$ Hz, H-2′ of glucose) and 4.42 (H-3β of aglycone). These findings support that glucose was attached to the C-3 position. The α-configuration of the anomeric carbon of glucopyranosyl unit was determined by $J_{H1\text{-}H2}$ value

(>7.0 Hz). Upon acid hydrolysis of **1** with 2.0 N HCl, glucose was detected on thin layer chromatography in the hydrolysed product. Accordingly, **1** was established as 1 β-*O*-acetyl-spirost-5,25(27)-dien-3α-yl-*O*-β-D-glucopyranoside, namely tupichinin B.

Tupichinin C (**2**) was obtained as colourless oil, $[\alpha]_D^{24}$-22° ($c = 0.004$, MeOH), and showed in the HRFABMS (positive mode) a pseudomolecular $[M + Na]^+$ peak at *m/z* 649.3668 (calcd 649.3666), consistent with the molecular formula $C_{33}H_{54}O_{11}$, suggesting a spirostanol saponin skeleton with seven degrees of unsaturation.

Unambiguous assignments for the ^{1}H and ^{13}C NMR signals were based on an analysis of the combination of DEPT, ^{1}H–^{1}H COSY, NOESY and HETCOR spectral data.

In the ^{1}H NMR spectrum in CD_3OD of **2**, signals characteristic of the spirostanol saponin skeleton were observed. The ^{1}H NMR spectrum showed signals for two tertiary methyl groups at δ 0.79 (3H, s, Me-18) and δ 1.31 (3H, s, Me-19), a secondary methyl group at δ 0.98 (3H, d, $J = 6.8$ Hz, Me-21) and a secondary methyl group at δ 1.08 (3H, d, $J = 7.2$ Hz, Me-27). Three oxymethine proton resonances at δ 3.76 (1H, br s), 3.70 (1H, br s) and 4.15 (1H, br s) were assigned to H-1α, H-2α and H-3α, respectively. Two methylene proton resonances at δ 2.46 (1H, dd, $J = 16.0$, 3.0 Hz) and 2.29 (1H, d, $J = 16.0$ Hz) were assigned to H-4α and H-4β, respectively. Besides, an oxymethine proton resonance at δ 4.42 (1H, q, $J = 7.2$ Hz) was assigned to H-16. Two oxymethine proton resonances at δ 3.28 (1H, d, $J = 10.8$ Hz) and 3.92 (1H, dd, $J = 10.8$, 2.4 Hz) were assigned to H-26β-eq and H-26α-ax, respectively. The anomeric proton resonance appeared at δ 4.75 (1H, d, $J = 7.6$ Hz). The fully decoupled ^{13}C and DEPT NMR spectra of **2** exhibited 33 carbon signals, which consisted of 4 methyls, 10 methylenes, 15 methines and 4 quaternary carbons. When the ^{13}C signals of **2** were compared with those of tupichigenin F (Pan et al. 2000a, 2000b), a set of additional six signals corresponding to a β-D-glucopyranosyl unit appeared. Four methyl groups at δ 16.8 (C-18), 13.6 (C-19), 14.7 (C-21) and 16.4 (C-27) were confirmed in ^{13}C NMR spectra, while the ^{13}C signal of C-27 resonance at the upper field gave evidence for the 25*S* configuration of **2**. Three signals at δ 56.9 (CH), 82.2 (CH) and 63.4 (CH) were assigned to the C-14, C-16 and C-17 positions, respectively. Two signals at δ 111.1 (C) and 66.1 (CH_2) were the characteristic resonances of C-22 and C-26, respectively. Furthermore, four signals at δ 78.6 (CH), 68.4 (CH), 71.4 (CH) and 84.1 (C) were assigned to the C-1, C-2, C-3 and C-5 positions, respectively. In the NOESY spectrum (Figure S2), the anomeric proton signal at δ 4.75 (H-1′ of glucose) showed correlations with the proton signal at δ 2.29 (H-4β of aglycone). The oxymethine proton signal at δ 3.76 (H-1 of aglycone) showed correlations with the proton signals at δ 1.31 (Me-19 of aglycone) and 1.45 (H-11α of aglycone). The oxymethine proton signal at δ 4.15 (H-3 of aglycone) showed correlations with the proton signals at δ 3.70 (H-2 of aglycone), 2.46 (H-4α of aglycone) and 2.29 (H-4β of aglycone). Furthermore, there were correlations between signals at δ 3.28 (H-26β of aglycone) and signals at δ 3.92 (H-26α of aglycone) and 1.08 (Me-27 of aglycone), respectively, in the NOESY spectrum. Upon acid hydrolysis of **2** with 2.0 N HCl, glucose was detected on thin layer chromatography in the hydrolysed mixture. Based on the above spectroscopic evidence, the structure of **2** was identified as (25*S*)-1β,2β,5β-trihydroxy-spirostane-3β-yl-*O*-β-D-glucopyranoside, namely tupichinin C.

Tupichinin D (**3**) was obtained as colourless plate, $[\alpha]_D^{24} + 59°$ (c 0.002, $CHCl_3$), and showed in the HRFABMS (positive mode) a pseudomolecular $[M + Na]^+$ peak at *m/z* 603.3512 (calcd 603.3509), consistent with the molecular formula $C_{32}H_{52}O_9$, suggesting a spirostanol saponin skeleton with seven degrees of unsaturation.

Unambiguous assignments for the ^{1}H and ^{13}C NMR signals were based on an analysis of the combination of DEPT, ^{1}H–^{1}H COSY, NOESY and HETCOR spectral data.

In the ^{1}H NMR spectrum in pyridine-d_5 of **3**, signals characteristic of the spirostanol saponin skeleton were observed. The ^{1}H NMR spectrum showed signals for two tertiary methyl groups at δ 0.79 (3H, s, Me-18) and δ 1.12 (3H, s, Me-19), a secondary methyl group at δ 0.98

(3H, d, $J = 7.2$ Hz, Me-21) and a secondary methyl group at δ 1.08 (3H, d, $J = 7.2$ Hz, Me-27). Three oxymethine proton resonances at δ 3.86 (1H, br s), 3.74 (1H, t, $J = 2.4$ Hz) and 4.20 (1H, br s) were assigned to H-1α, H-2α and H-3α, respectively. The oxymethine proton resonance at δ 4.38 (1H, m) was assigned to H-16. Two oxymethine proton resonances at δ 3.20 (1H, t, $J = 11.6$ Hz) and 3.84 (1H, dd, $J = 11.6$, 5.6 Hz) were assigned to H-26β-eq and H-26α-ax, respectively. The anomeric proton resonance appeared at δ 4.41 (1H, d, $J = 7.2$ Hz). The fully decoupled ^{13}C and DEPT NMR spectra of **3** exhibited 32 carbon signals, which consisted of 4 methyls, 10 methylenes, 15 methines and 3 quaternary carbons.

Four methyl groups at δ 16.9 (C-18), 19.2 (C-19), 14.7 (C-21) and 16.4 (C-27) were confirmed in ^{13}C NMR spectra. Three signals at δ 57.2 (CH), 82.3 (CH) and 63.7 (CH) were assigned to the C-14, C-16 and C-17 positions, respectively. Two signals at δ 111.1 (C) and 67.0 (CH_2) were the characteristic resonances of C-22 and C-26, respectively. Furthermore, three signals at δ 78.5 (CH), 75.2 (CH) and 71.1 (CH) were assigned to the C-1, C-2 and C-3 positions, respectively. In the NOESY spectrum (Figure S3), the anomeric proton signal at δ 4.41 (H-1′ of xylose) showed correlations with the proton signals at δ 3.74 (H-2 of aglycone) and 4.20 (H-3 of aglycone). The oxymethine proton signal at δ 3.74 (H-2 of aglycone) showed correlations with the proton signals at δ 3.86 (H-1 of aglycone) and 4.20 (H-3 of aglycone). Moreover, there was correlation between signal at δ 3.20 (H-26β of aglycone) and signal at δ 1.08 (Me-27 of aglycone). When **3** was hydrolysed with 2.0 N HCl, only xylose was detected in the hydrolysate on TLC. Thus, **3** was identified as (25*S*)-1β,2β-dihydroxy-5β-spirostane-3β-yl-*O*-β-D-xylopyranoside, namely tupichinin D.

3. Experimental

3.1. *General*

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), DEPT, HETCOR, COSY, NOESY and long-range HETCOR spectra were obtained on a Varian NMR spectrometer (Unity Plus). Chemical shifts (δ) were reported in ppm relative to residual solvent signals. The multiplicities of ^{1}H signals are designated by the following abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; br = broad and m = multiplet. All coupling constants, *J*, are reported in Hertz. ^{13}C NMR spectra were acquired on a broad band decoupled mode, and the multiplicities were obtained using DEPT sequences. Optical rotations were measured with a JASCO DIP-370 digital polarimeter (Japan). Low-resolution FABMS was collected on a JEOL JMS-SX/SX 102A mass spectrometer (Tokyo, Japan) or a Quattro GC/MS spectrometer (USA) with a direct inlet system. High-resolution FABMS were measured on a JEOL JMS-HX 110 mass spectrometer (Tokyo, Japan). Silica gel 60 (Merck, 230–400 mesh, Darmstadt, Germany) was used for column chromatography, precoated silica gel plates (Merck, Kieselgel60 F-254, 0.20 mm) were used for analytical TLC and precoated silica gel plates (Merck, Kieselgel60 F-254, 0.50 mm) were used for preparative TLC. The spots were detected by spraying with 50% H_2SO_4 followed by heating on a hot plate.

3.2. *Plant material*

The rhizomes of *T. chinensis* were purchased in Tainan, Taiwan. A voucher specimen (No. 20120209) was deposited in the Department of Applied Chemistry and Materials Science, Fooyin University, Kaohsiung, Taiwan.

3.3. *Extraction and isolation*

The air-dried rhizomes of *T. chinensis* (3 kg) were extracted repeatedly with MeOH at room temperature. After evaporation of the methanol under vacuum, the residue was suspended in

water and then partitioned successively to yield *n*-hexane (64.6 g), $CHCl_3$ (19.5 g) and EtOAc (11.4 g) fractions. The extraction and the isolation processes were carried out under the neutral conditions. The EtOAc fraction (11.4 g) of *T. chinensis* was loaded onto the silica gel column and subjected to gradient elution with $CHCl_3$–MeOH mixtures of increasing polarity to yield 12 fractions. The fraction obtained by elution with $CHCl_3$:MeOH (10:1) was subjected to rechromatography on silica gel elution with $CHCl_3$:MeOH (100:8) to afford compound **1** (25 mg) and the fraction obtained by elution with $CHCl_3$:MeOH (10:2) was subjected to rechromatography on silica gel elution with $CHCl_3$:MeOH (100:15) to afford compound **2** (28 mg). The fraction eluted from $CHCl_3$:MeOH (100:5) was rechromatographed on silica gel elution with $CHCl_3$:MeOH (100:4) to afford compound **3** (15 mg).

3.4. *Tupichinin B (1)*

White solid, $[\alpha]_D^{24}$ −18° (*c* 0.004, MeOH). FAB-MS (positive mode) *m/z*: 655 $[M + Na]^+$. ^1H NMR (400 MHz, pyridine-d_5) δ: 0.88 (3H, s, H-18), 1.14 (3H, d, $J = 7.0$ Hz, H-21), 1.22 (3H, s, H-19), 2.08 (3H, s, 1-*O*-Ac), 4.11 (1H, d, $J = 12.0$ Hz, H-26β), 4.42 (1H, br s, H-3), 4.50 (1H, d, $J = 12.0$ Hz, H-26 α), 4.55 (1H, q-like, $J = 7.0$ Hz, H-16), 4.86 (1H, s, H-27_A), 4.89 (1H, s, H-27_B), 4.98 (1H, d, $J = 7.5$ Hz,H-1′), 5.53 (1H, d, $J = 4.8$ Hz,H-1), 5.67 (1H, d, $J = 4.8$ Hz, H-6) and ^{13}C NMR (100 MHz, pyridine-d_5) δ: 78.9 (d, C-1), 33.9 (t, C-2), 75.2 (d, C-3), 39.0 (t, C-4), 138.3 (s, C-5), 126.7 (d, C-6), 32.5 (t, C-7), 33.0 (d, C-8), 50.0 (d, C-9), 43.6 (s, C-10), 24.4 (t, C-11), 40.8 (t, C-12), 40.7 (s, C-13), 57.2 (d, C-14), 32.9 (t, C-15), 82.2 (d, C-16), 63.7(d, C-17), 17.2 (q, C-18), 14.9 (q, C-19), 42.6 (d, C-20), 15.8 (q, C-21), 110.5 (s, C-22), 33.9 (t, C-23), 29.7 (t, C-24), 145.0 (s, C-25), 65.8 (t, C-26), 109.9 (t, C-27), 104.2 (d, C-1′), 75.7 (d, C-2′), 79.1 (d, C-3′), 72.4 (d, C-4′), 79.1 (d, C-5′), 63.5 (t, C-6′), 171.5 (s, 1-*O*-$\underline{CO}CH_3$), 22.6 (q, 1-*O*-$CO\underline{CH_3}$). HRFABMS *m/z* 655.3563 $[M + Na]^+$(calcd for $C_{35}H_{52}O_{10}Na$ $\overline{655}$.3560).

3.5. *Tupichinin C (2)*

Colourless oil, $[\alpha]_D^{24}$ −22°(*c* 0.004, MeOH). FAB-MS (positive mode) *m/z* 649 $[M + Na]^+$. ^1H NMR (400 MHz,CD_3OD) δ: 0.79 (3H, s, Me-18),0.98 (3H, d, $J = 6.8$ Hz, Me-21), 1.08 (3H, d, $J = 7.2$ Hz, Me-27), 1.31 (3H, s, Me-19), 1.45 (2H, m, H-11), 2.29 (1H, d, $J = 16.0$ Hz, H-4β), 2.46 (1H, dd, $J = 16.0$, 3.0 Hz, H-4α), 3.28 (1H, d, $J = 10.8$ Hz, H-26β-eq), 3.70 (1H, br s, H-2α), 3.71 (1H, dd, $J = 11.6$, 4.0 Hz, H-6_B′), 3.76 (1H, br s, H-1α), 3.85 (1H, d, $J = 11.6$ Hz, H-6_A′), 3.92 (1H, dd, $J = 10.8$, 2.4 Hz, H-26α-ax), 4.15 (1H, br s, H-3α), 4.42 (1H, q, $J = 7.2$ Hz, H-16), 4.75 (1H, d, $J = 7.6$ Hz, H-1′) and ^{13}C NMR (100 MHz, CD_3OD) spectral data δ: 78.6 (d, C-1), 68.4 (d, C-2), 71.4 (d, C-3), 36.4 (t, C-4), 84.1 (s, C-5), 31.1 (t, C-6), 29.7 (t, C-7), 35.5 (d, C-8), 46.4 (d, C-9), 47.2 (s, C-10), 22.1 (t, C-11), 40.8 (t, C-12), 41.3 (s, C-13), 56.9 (d, C-14), 32.7 (t, C-15), 82.2 (d, C-16), 63.4(d, C-17), 16.9 (q, C-18), 13.6 (q, C-19), 43.4 (d, C-20), 14.7 (q, C-21), 111.1 (s, C-22), 27.0 (t, C-23), 26.7 (t, C-24), 28.5 (d, C-25), 66.1 (t, C-26), 16.4 (q, C-27), 97.2 (d, C-1′), 75.4 (d, C-2′), 78.2 (d, C-3′), 70.9 (d, C-4′), 77.9 (d, C-5′), 62.0 (t, C-6′). HRFABMS *m/z* 649. 3668 $[M + Na]^+$ (calcd for $C_{33}H_{54}O_{11}Na$ 649. 3666).

3.6. *Tupichinin D (3)*

Colourless plate, $[\alpha]_D^{24}$ + 59° (*c* 0.002, $CHCl_3$). FAB-MS (positive mode) *m/z* 603 $[M + Na]^+$. ^1H NMR (400 MHz, pyridine-d_5) δ: 0.79 (3H, s, Me-18), 0.98 (3H, d, $J = 7.2$ Hz, Me-21), 1.08 (3H, d, $J = 7.2$ Hz, Me-27), 1.12 (3H, s, Me-19), 1.98 (1H, m, H-5), 3.20 (1H, t, $J = 11.6$ Hz, H-26β-eq), 3.27 (1H, d, $J = 8.8$ Hz, H-5_B′), 3.28 (1H, m, H-2′), 3.34 (1H, t, $J = 10.2$ Hz, H-3′), 3.50 (1H, td, $J = 10.4$, 5.6 Hz, H-4′), 3.74 (1H, t, $J = 2.4$ Hz, H-2α), 3.84 (1H, dd, $J = 11.6$, 5.6 Hz, H-26α-ax), 3.86 (1H, br s, H-1α), 3.92 (1H, dd, $J = 10.8$, 2.4 Hz, H-5_A′), 4.20 (1H, br s,

H-3α), 4.38 (1H, m, H-16), 4.41 (1H, d, $J = 7.2$ Hz, H-1′) and ^{13}C NMR (100 MHz, pyridine-d_5) δ: 78.5 (d, C-1), 75.2 (d, C-2), 71.1 (d, C-3), 34.0 (t, C-4), 31.6 (d, C-5), 27.3 (t, C-6), 26.5 (t, C-7), 36.8 (d, C-8), 43.0 (d, C-9), 42.5 (s, C-10), 21.8 (t, C-11), 41.1 (t, C-12), 41.5 (s, C-13), 57.2 (d, C-14), 32.7 (t, C-15), 82.3 (d, C-16), 63.7 (d, C-17), 16.9 (q, C-18), 19.2 (q, C-19), 43.4 (d, C-20), 14.7 (q, C-21), 111.1 (s, C-22), 27.0 (t, C-23), 26.7 (t, C-24), 28.5 (d, C-25), 67.0 (t, C-26), 16.4 (q, C-27), 103.8 (d, C-1′), 75.0 (d, C-2′), 77.6 (d, C-3′), 71.2 (d, C-4′), 66.1 (t, C-5′). HRFABMS *m/z* 603.3512 $[M + Na]^+$ (calcd for $C_{32}H_{52}O_9Na$ 603.3509).

3.7. *Acid hydrolysis*

Compounds **1** (5 mg), **2** (4 mg) and **3** (4 mg) were hydrolysed with 2.0 mol/L HCl. The hydrolysis and the detection of sugars in hydrolysed products were carried out according to the procedures described in the literature (Zou et al. 2005).

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S21.

Acknowledgements

The authors gratefully acknowledge the Department of Applied Chemistry and Materials Science, Fooyin University, Taiwan for financial support.

References

Cai J, Zhu ZG, Yu CL, Lei LS, Wu SG. 2007. Saponin from *Tupistra chinensis* Bakerinhibits mouse sarcoma S2180 cell proliferation *in vitro* and implanted solid tumor growth in mice. J South Med Univ. 27:188–194.

Guo ZY, Zou K, Wang JZ, Liu C, Tang ZC, Yang CY. 2009. Structural elucidation and NMR spectral assignment of three new furostanol saponins fromthe roots of *Tupistra chinensis*. Magn Reson Chem. 47:613–616.

Hang JL, Li H. 1990. Study on the taxonomic system of the genus *Tupistra*. Acta Bot Yunnan Suppl. III:49–61.

Jiangsu New Medical College. 1985. Traditional Chinese medicine dictionary. Shanghai: Shanghai Scientific Technology Press. p. 907.

Liu CX, Guo ZY, Xue YH, Cheng J, Huang NY, Zhou Y, Cheng F, Zou K. 2012. Five new furostanol saponins from the rhizomes of *Tupistra chinensis*. Fitoterapia. 83:323–328.

Liu CX, Guo ZY, Xue YH, Zhang HY, Zhang HQ, Zou K, Huang NY. 2012. Tupisteroide A-C, three new polyhydroxylated steroidal constituents from the roots of *Tupistra chinensis*. Magn Reson Chem. 50:320–324.

Pan WB, Chang FR, Wu YC. 2000a. Tupichigenin A, a new steroidal sapogenin from *Tupistra chinensis*. J Nat Prod. 63:861–863.

Pan WB, Chang FR, Wu YC. 2000b. Spirostanol sapogenins from the underground parts of *Tupistra chinensis*. Chem Pharm Bull. 48:1350–1353.

Pan WB, Chang FR, Wei LM, Wu YC. 2003. New flavans, spirostanol sapogenins, and a pregnane genin from *Tupistra chinensis* and their cytotoxicity. J Nat Prod. 66:161–168.

Pan WB, Wei LM, Wei LL, Wu YC. 2006. Chemical constituents of *Tupistra chinensis* rhizomes. Chem Pharm Bull. 54:954–958.

Pan ZH, Li Y, Liu JL, Ning DS, Li DP, Wu XD, Wen YX. 2012. A cytotoxic cardenolide and a saponin from the rhizomes of *Tupistra chinensis*. Fitoterapia. 83:1489–1493.

Wu GX, Wei XY, Chen WX. 2005. Spirostane steroidal saponins from the underground parts of *Tupistra chinensis*. Chin Chem Lett. 16:911–914.

Xu LL, Zou K, Wang JZ, Wu J, Zhou Y, Dan FJ, Yang J. 2007. New polyhydroxylated furostanol saponins with inhibitory action against NO production from *Tupistra chinensis*. Molecules. 12:2029–2037.

Yang QX, Zhang YL, Li HZ, Yang CR. 2005. Polyhydroxylated steroidal constituents from the fresh rhizomes of *Tupistra yunnanensis*. Steroids. 70:732–737.

Zhan YH. 1994. China Shenongjia resources of medicinal plants. Wuhan: Hubei Scientific and Technological Press.

Zou K, Wang JZ, Du M, Li Q, Tu G. 2006. A pair of diastereoisomeric steroidal saponins from cytotoxic extracts of *Tupistra chinensis* rhizomes. Chem Pharm Bull. 54:1440–1442.

Zou K, Wang JZ, Wu J, Zhou Y, Liu C, Dan FJ, Zhang YX, Yang J. 2007. Furostanol saponins with inhibitory action against COX-2 production from *Tupistra chinensis* rhizomes. Chin Chem Lett. 18:1239–1242.

Zou K, Tong WY, Liang H, Cui JR, Tu GZ, Zhao YY, Zhang RY. 2005. Diastereoisomeric saponins from *Albizia julibrissin*. Carbohydr Res. 340:1329–1334.

Zou K, Wu J, Du M, Liu C, Tu GZ, Wang JZ. 2007. Diastereoisomeric saponins from therhizomes of *Tupistra chinensis*. Chin Chem Lett. 18:65–68.

Zou K, Wang JZ, Guo ZY, Du M, Wu J, Zhou Y, Dan FJ, Liu C. 2009. Structural elucidation of four new furostanol saponins from *Tupistra chinensis* by 1D and 2D NMR spectroscopy. Magn Reson Chem. 47:87–91.